# Analysis of the Volatile Components of Five Turkish *Rhododendron* Species by Headspace Solid-Phase Microextraction and GC-MS (HS-SPME-GC-MS)

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Volatile constituents of various solvent extracts (*n*-hexane, $CH_2Cl_2$, $H_2O$) of 15 different organs (leaves, flowers, fruits) of five *Rhododendron* species (Ericaceae) growing in Turkey were trapped with headspace solid-phase microextraction (HS-SPME) technique and analyzed by GC-MS. A total of 200 compounds were detected and identified from organic extracts, while the water extracts contained only traces of few volatiles. The $CH_2Cl_2$ extract of the *R. luteum* flowers was found to exhibit the most diverse composition: 34 compounds were identified, with benzyl alcohol (16.6%), limonene (14.6%) and *p*-cymene (8.4%) being the major compounds. The $CH_2Cl_2$-solubles of *R. x sochadzeae* leaves contained only phenyl ethyl alcohol. This study indicated appreciable intra-specific variations in volatile compositions within the genus. Different anatomical parts also showed altered volatile profiles. This is the first application of HS-SPME-GC-MS on the volatiles of *Rhododendron* species.

*Key words: Rhododendron*, Headspace Solid-Phase Microextraction, HS-SPME-GC-MS

## Introduction

Volatile compounds are diverse in the plant kingdom and play a complex, vital role in relationships between plants and their ecological environments. Various conventional methods have been used for extracting fragrances of aromatic herbs and flowers for commercial and research purposes. Some of these methods include distillation, solvent extraction, supercritical solvent ($CO_2$) extraction and headspace trapping. Solid-phase microextraction (SPME) offers an alternative sampling technique for the analysis of volatile organics (Rohloff, 1999). This method is based on the adsorption of analytes on a polymeric stationery phase deposited on a fused-silica fiber via a partitioning effect between the adsorbent and the sample matrix. The adsorption is provided by immersing the precoated fiber in a liquid sample or exposing it to the headspace above a liquid or solid sample. SPME can be coupled with GC or GC-MS, where the adsorbed analytes are thermally desorbed in the injection port of the gas chromatograph with subsequent transfer to a capillary column (Bicchi *et al.*, 2000). Although developed for the analysis of water pollutants originally, SPME has found wide application in several other fields, particularly in food chemistry and pharmaceutical analyses (Rohloff, 1999).

*Rhododendron* species (mountain laurel) are deciduous or evergreen shrubs commonly used as garden plants worldwide. Six *Rhododendron* species, one of which (*R. smirnovii*) is endemic, grow naturally in Turkey, especially in the northeastern Anatolia (Black Sea region) (Stevens, 1978). Some members of this genus, such as *R. ponticum* and *R. luteum* are well known for being poisonous (Baytop, 1999; Onat *et al.*, 1991; Sütlüpınar *et al.*, 1993). The consumption of "mad honey" (deli bal in Turkish) produced from the nectar of these plants still causes intoxications in humans in the eastern Black Sea region of Turkey (Baytop, 1999; Onat *et al.*, 1991; Sütlüpınar *et al.*, 1993). Serious *Rhododendron* poisonings are also common in livestock, particularly in sheep and goats fed with the young leaves or flowers of these species (Baytop, 1999; Puschner *et al.*, 2001). The toxic effects

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of these plants have been attributed to grayanine-type tetracyclic diterpenes (grayanatoxins = andromedotoxins) that bind to sodium channels in cell membranes to increase the permeability of sodium ions in excitable membranes (Onat *et al.*, 1991; Sütlüpınar *et al.*, 1993). Interestingly, toxic *Rhododendron* species, particularly *R. ponticum*, are common folk medicines of the Black Sea region. *R. ponticum* is widely used as analgesic for the treatment of rheumatic or dental pain, common colds and edema, both internally and externally (Baytop, 1999). The other Turkish *Rhododendron* species have no reputation for being toxic. Instead, flowers of some species are eaten or their nectars are sucked by the local people (Stevens, 1978; D. T. personal observation).

The main objective of this study is the rapid identification of the volatile constituents of the solvent extracts prepared from the leaves and the flowers of two toxic *Rhododendron* species, *R. ponticum* and *R. luteum*, using HS-SPME coupled with GC-MS. Furthermore, 11 available organs (leaves, flowers or fruits) of three other *Rhododendron* plants were also studied using the same experimental conditions for comparison. Although SPME has been employed for the rapid extraction and analysis of some plant volatiles, there is no report on the application of HS-SPME on this genus. This study showed HS-SPME coupled with GC-MS to be a useful tool for the quick screening of both major and minor aromatic constituents of the organic extracts obtained from five Turkish *Rhododendron* species.

## Materials and Methods

### *Plant material*

Plants were collected in early July 2001 from different locations in northeast Anatolia, Turkey, and identified by one of us (A. A. D.). Identification of the specimens was based on the account of the *Rhododendron* in Flora of Turkey (Stevens, 1978). Voucher specimens were deposited at HUB, Department of Biology (Faculty of Science) and/or Herbarium of Department of Pharmacognosy (Faculty of Pharmacy) of Hacettepe University. Table I illustrates the names, voucher numbers, collection sites and the parts of the plants investigated.

### *Extraction and partition*

Shadow-dried plant material (10–200 g dry weight) was ground and extracted at room temperature with *n*-hexane (extract no. 1), $CH_2Cl_2$ (extract no. 2) and $H_2O$ (extract no. 3) (three times each), respectively. Due to very low amount of material, the flowers of *R. ponticum*, *R. luteum* and *R. ungernii* were only extracted with $CH_2Cl_2$ and then with $H_2O$. The organic solvents were removed under low pressure at room temperature, while the water extracts were freeze-dried. Table I shows the extracts prepared from different plant samples and the abbreviations used for these extracts in the body text.

### *Headspace-SPME*

The manual SPME device (Supelco, Bellafonte, PA, USA) with a fibre precoated of a 65 $\mu$m thick layer of polydimethylsiloxane/divinylbenzene (PDMS/DVB-blue) was used for extraction of the plant volatiles. The vial containing the plant extract was sealed with parafilm. The fiber was pushed through the film layer for exposure to the headspace of the extract for 15 min at 50 °C. The fiber was then inserted immediately into the injection port of the GC-MS for the desorption of the adsorbed volatile compounds for analysis. This procedure was applied in triplicate.

### *Analysis of volatile compounds*

The volatiles were analyzed by GC-MS using a Hewlett Packard GCD system. An HP-Innowax FSC column (60 m × 0.25 mm inner diameter, with 0.25 mm film thickness) was used with helium as carrier gas (1 ml/min). GC oven temperature was kept at 60 °C for 10 min and programmed to 220 °C at a rate of 4 °C/min, then kept constant at 220 °C for 10 min and then programmed to 240 °C at a rate of 1 °C/min, at splitless mode. The injector temperature was at 250 °C. EI-mass spectra were recorded at 70 eV. Mass range was from 35 to 425 *m/z*.

### *Identification of compounds*

Individual components were identified by comparison of their mass spectra using both "Medicinal and Aromatic Plant and Drug Research Centre (TBAM) Library of Essential Oil Constitu-

ents" and "Wiley GC-MS Library". Relative percentage amounts of the separated compounds were calculated automatically from peak areas of the total ion chromatograms (TIC). *n*-Alkanes were used as reference points in the calculation of relative retention indices (RRI). The volatile compounds identified are listed in Table II (*n*-hexane-solubles) and Table III ($CH_2Cl_2$-solubles).

## Results and Discussion

The current study aimed the determination of the HS-SPME volatile profile of the flowers and the leaves of toxic *R. ponticum* and *R. luteum* that apparently attract the honeybees to collect their nectar for the production of mad honey. In addition, the leaves (*R. x sochadzeae*, *R. ungernii* and *R. smirnovii*), the flowers (*R. ungernii*) and the unripe fruits (*R. x sochadzeae*) of three other *Rhododendron* species were also collected in their native regions (Table I). Since we were unable to perform a simultaneous on-site field sampling of living plants and subsequent GC-MS analysis, alternately we tried to extract the volatiles of the shadow-dried and coarsely ground plant samples by HS-SPME. This first attempt however, turned out to be unsuccessful. Minute amounts of material (especially the flowers) and the absence of sharp odor to carry out a distillation process tempted us to perform a solvent extraction. For the extraction of flowers, $CH_2Cl_2$ was chosen, as it is highly volatile and capable of dissolving a wide class of flavors and volatiles (Ceva-Antunes *et al.*, 2003). All other plant material was extracted first with *n*-hexane and subsequently with $CH_2Cl_2$ and $H_2O$. Following the HS-SPME collection of the volatile compounds, GC-MS was used to identify the analytes in the headspace needle. $H_2O$ extracts were found to be very poor in volatiles and only traces of some organics were detected. Therefore, the results will not be discussed here. A total of 200 substances were identified in the organic extracts, which showed both qualitative and quantitative differences in a relative sense. Eighty-seven compounds in total were characterized in the *n*-hexane extracts and 115 in the $CH_2Cl_2$ extracts.

The headspace of the *n*-hexane extract of *R. ponticum* leaves (RPL-1) contained 14 volatile compounds, accounting 97.0% of the whole volatiles. The principal constituent of this extract was tentatively identified as a tricyclic diterpene, 5,15-rosadiene (42.8%), followed by 2-ethyl-hexanol (13.3%) and styrene (10.0%). Only 8 compounds were characterized from the $CH_2Cl_2$ extract of the leaves (RPL-2), with 1-butanol (17.0%) as the major component. The other major components detected in the same sample were $\gamma$-butyrolactone (13.5%), styrene (11.8%), benzyl alcohol (11.7%) and (*Z*)-3-hexenol (10.0%). The living flowers of *R. ponticum* had an attractive purplish-pink color with a slight fragrance. However, 1-methyl-2-pyrrolidone, a volatile with an unpleasant odor, was also tentatively determined to be the most abundant (79.7%) constituent of the whole volatile fraction (95.6%) of *R. ponticum* flowers. This may indicate that the odor is more complex, with a strong influence from the other minor volatiles that have influence on the natural scent of the flowers. Surprisingly, 1-methyl-2-pyrrolidone was completely absent in the organic extracts of the leaves.

Table I. Turkish *Rhododendron* species examined by HS-SPME coupled with GC-MS.

| Plant species | Organ(s) used | Extracts | Voucher no | Collection site |
|---|---|---|---|---|
| *R. ponticum* L. | Leaves (RPL)<br>Flowers (RPF) | 1, 2, 3<br>2, 3 | AAD-9881 | Artvin: Damar village, Murgul, 1300 m |
| *R. luteum* Sweet | Leaves (RLL)<br>Flowers (RLF) | 1, 2, 3<br>2, 3 | AAD-9882 | Artvin: Murgul, Damar village, 1300 m |
| *R. x sochadzeae* Charadze & Davlianidze | Leaves (RSoL)<br>Fruits (RSoFr) | 1, 2, 3<br>1, 2, 3 | AAD-9892 | Artvin: Tiryal mountain, Taslıca village, 2285 m |
| *R. ungernii* Trautv. | Leaves (RUL)<br>Flowers (RUF) | 1, 2, 3<br>2, 3 | AAD-9880 | Artvin: Murgul, *Picea orientalis* forest, 1626 m |
| *R. smirnovii* Trautv. | Leaves (RSL) | 1, 2, 3 | AAD-9889 | Artvin: Tiryal mountain, Taslıca village, 2285 m |

1: Hexane extract; 2: $CH_2Cl_2$ extract; 3: $H_2O$ extract.

Table II. The volatiles identified from the *n*-hexane extracts of Turkish *Rhododendron* species by SPME-GC-MS. RPL, *R. ponticum* leaves; RLL, *R. luteum* leaves; RSoL, *R. x sochadzeae* leaves; RSoFr, *R. x sochadzeae* fruits; RUL, *R. ungernii* leaves; RSL, *R. smirnovii* leaves; 1, Hexane extract.

| RRI | Compound | RPL-1 | RLL-1 | RSoL-1 | RSoFr-1 | RUL-1 | RSL-1 |
|---|---|---|---|---|---|---|---|
| 893 | Ethyl acetate | 4.4 | 13.3 | 4.5 | 44.8 | 4.4 | |
| 1000 | Decane | | 0.8 | tr | 3.5 | | |
| 1093 | Hexanal | | 1.3 | 4.4 | 4.5 | | |
| 1100 | Undecane | 4.0 | 6.9 | 6.0 | 1.9 | | |
| 1155 | 1-Butanol | 3.3 | | | | 5.8 | |
| 1272 | Styrene | 10.0 | 4.3 | 7.9 | 11.7 | | |
| 1348 | 6-Methyl-5-hepten-2-one | 3.2 | 11.1 | 11.9 | 3.0 | 29.4 | 21.7 |
| 1360 | Hexanol | 1.5 | 1.6 | | | | |
| 1391 | (*Z*)-3-Hexenol | 4.5 | 1.6 | 1.0 | 1.9 | 6.4 | 11.4 |
| 1400 | Tetradecane | | | | | 4.8 | |
| 1450 | *trans*-Linalool oxide (Furanoid) | | 1.5 | 4.1 | | | |
| 1452 | 1-Octen-3-ol | 3.1 | 4.0 | 2.0 | | 7.9 | 9.6 |
| 1478 | *cis*-Linalool oxide (Furanoid) | | 1.5 | | | | |
| 1479 | (*E*,*Z*)-2,4-Heptadienal | | | 5.3 | 2.3 | | |
| 1496 | 2-Ethyl-hexanol | 13.3 | 7.1 | 4.8 | 2.2 | 24.4 | 10.1 |
| 1522 | 3,5-Octadien-2-one | | 1.9 | 3.1 | 4.5 | | |
| 1553 | Linalool | | 2.4 | | | | |
| 1602 | 6-Methyl-3,5-heptadien-2-one | 2.5 | 3.4 | 3.0 | | 7.0 | 14.6 |
| 1610 | Calarene (= $\beta$-gurjunene) | | | 7.1 | 9.0 | | |
| 1641 | Methyl benzoate | | | 2.3 | | | |
| 1661 | Alloaromadendrene | | | 5.1 | | | |
| 1685 | Ethyl benzoate | | | 1.6 | | | |
| 1706 | $\alpha$-Terpineol | 2.0 | 6.6 | | | | |
| 1763 | Naphthalene | 1.9 | 1.6 | 1.2 | | | |
| 1868 | (*E*)-Geranyl acetone | | 1.3 | 1.4 | | 4.4 | |
| 1896 | Benzyl alcohol | 0.5 | 1.6 | 6.35.4 | | | |
| 1908 | Unknown* | | 0.3 | 2.4 | | | |
| 1937 | Phenylethyl alcohol | | 1.2 | 1.0 | 2.4 | 5.5 | 7.4 |
| 2104 | Viridiflorol | | | | | | 15.4 |
| 2232 | 5,15-Rosadiene** | 42.8 | | | | | |
| | Total | 97.0 | 75.3 | 86.4 | 91.7 | 100 | 95.6 |

RRI: Relative retention indices calculated against *n*-alkanes, % calculated from TIC data.
tr: Trace (< 0.1%).
* Unknown; EIMS (70 eV): *m/z* (rel. int.) = 162 (6.9), 161 (13.4), 145 (96.2), 130 (14.8), 127 (15.1), 113 (27.9), 101 (35.8), 74 (15.4), 71 (100), 59 (20.1), 43 (76.1).
** Tentative identification from Wiley.

The volatile profile of the *n*-hexane-solubles of the second toxic *Rhododendron* species, *R. luteum*, was somewhat similar to that of *R. ponticum*, with notable differences in volatile components and their relative quantities. Ethyl acetate (13.3%), 6-methyl-5-hepten-2-one (11.1%), 2-ethyl-hexanol (7.1%) and $\alpha$-terpineol (6.6%) comprised the major volatile constituents of this extract (RLL-1). The $CH_2Cl_2$ extract of the same material (RLL-2) was strongly dominated by 1-butanol (58.7%), followed by benzyl alcohol (17.1%) and phenylethyl alcohol (6.7%), representing 82.5% of the volatile fraction (88.7%). The fresh yellow flowers of *R. luteum* as well as the $CH_2Cl_2$ extract prepared therefrom were remarkably odoriferous. Indeed, the $CH_2Cl_2$ extract (RLF-2) showed the most diverse composition of all *Rhododendron* extracts investigated here. Thirty-four volatile compounds were identified, with benzyl alcohol (16.6%), limonene (14.6%) and *p*-cymene (8.4%) being the major ones. The $H_2O$-soluble fraction of the flowers had also a light, delicate odor, very similar to that of $CH_2Cl_2$ extract. However, we were only able to detect trace amounts (< 0.1%) of limonene, 1,8-cineol and *p*-cymene in this extract (data not shown).

The volatile composition of the *n*-hexane extract of the natural hybrid, *R. x sochadzeae* (RSoL-1)

Table III. The volatiles identified from the $CH_2Cl_2$ extracts of Turkish *Rhododendron* species by SPME-GC-MS. RPL, *R. ponticum* leaves; RPF, *R. ponticum* flowers; RLL, *R. luteum* leaves; RLF, *R. luteum* flowers; RSoL, *R. x sochadzeae* leaves; RSoFr, *R. x sochadzeae* fruits; RUL, *R. ungernii* leaves; RUF, *R. ungernii* flowers; RSL, *R. smirnovii* leaves; 2, $CH_2Cl_2$ extract.

| RRI | Compound | RPL-2 | RPF-2 | RLL-2 | RLF-2 | RSoL-2 | RSoFr-2 | RUL-2 | RUF-2 | RSL-2 |
|---|---|---|---|---|---|---|---|---|---|---|
| 893 | Ethyl acetate | | | | | | 1.4 | 2.6 | 23.4 | 0.7 |
| 1032 | $\alpha$-Pinene | | | | 0.6 | | | | | |
| 1093 | Hexanal | | 3.7 | | 0.2 | | | 1.1 | 9.5 | |
| 1100 | Undecane | | | | 0.4 | | | | | |
| 1146 | $\delta$-2-Carene | | | | 0.6 | | | | | |
| 1155 | 1-Butanol | 17.0 | | 58.7 | | | 2.5 | | | |
| 1176 | $\alpha$-Phellandrene | | | | 1.2 | | | | | |
| 1197 | Methyl hexanoate | | | tr | | | | | | 0.9 |
| 1203 | Limonene | | | | 14.6 | | | | | |
| 1213 | 1,8-Cineole | | | | 5.8 | | | | | |
| 1255 | $\gamma$-Terpinene | | | | 0.6 | | | | | |
| 1260 | 1-Pentanol | | tr | | | | | | | |
| 1272 | Styrene | 11.8 | | | | | | | | |
| 1280 | *p*-Cymene | | | | 8.4 | | | | | |
| 1304 | 1-Octen-3-one | | 0.3 | | | | | | | |
| 1335 | (*E*)-2-Heptenal | | 0.2 | | | | | | | |
| 1348 | 6-Methyl-5-hepten-2-one | | | | 2.7 | | | | 17.7 | 1.4 |
| 1360 | Hexanol | | | | | | 1.2 | | | |
| 1391 | (*Z*)-3-Hexenol | 10.0 | | | | | 0.7 | | | 4.8 |
| 1395 | 2-Butoxy ethanol | 2.5 | | | | | | | | |
| 1399 | Methyl octanoate | | 0.2 | | | | | | | |
| 1400 | Tetradecane | | | | | | 1.4 | | | |
| 1400 | Nonanal | | 0.9 | | | | | | | |
| 1406 | $\alpha$-Fenchone | | | | 5.4 | | | | | |
| 1450 | *trans*-Linalool oxide (Furanoid) | | | | | | 1.9 | | | |
| 1452 | 1-Octen-3-ol | | 2.3 | | | | 0.6 | tr | 4.8 | |
| 1479 | (*E*,*Z*)-2,4-Heptadienal | | | | | | | 0.8 | | |
| 1483 | Octyl acetate | | | | 1.4 | | | | | |
| 1496 | 2-Ethyl-hexanol | | | | 3.4 | | 7.0 | 1.0 | 9.3 | |
| 1500 | Methyl nonanoate | | 0.3 | | | | | | | |
| 1507 | (*E*,*E*)-2,4-Heptadienal | | | | | | | 0.9 | | |
| 1541 | Benzaldehyde | | | | 1.3 | | | 0.9 | | |
| 1522 | 3,5-Octadien-2-one | | 3.9 | | | | 1.2 | | | |
| 1553 | Linalool | | | | 2.3 | | | | | |
| 1562 | Octanol | | | | 0.9 | | | | | |
| 1595 | Isothymol methyl ether | | | | 0.4 | | | | | |
| 1598 | Thymol methyl ether | | | | 0.6 | | | | | |
| 1602 | 6-Methyl-3,5-heptadien-2-one | | 0.6 | | 0.8 | | 1.1 | 2.2 | | 1.9 |
| 1621 | 2-Octen-1-ol | | 0.2 | | | | | | | |
| 1628 | 2-(2-Ethoxyethoxy) ethanol | | | 3.3 | 1.4 | | 2.2 | 12.8 | | 7.8 |
| 1651 | $\gamma$-Butyrolactone | 13.5 | | 2.9 | 0.5 | | 3.1 | 2.7 | | 6.0 |
| 1663 | Phenylacetaldehyde | | | | 0.5 | | | | | |
| 1678 | 1-Methyl-2-pyrrolidone* | | 79.7 | | | | | | | |
| 1706 | $\alpha$-Terpineol | | | | 1.2 | | | | | |
| 1715 | (*E*,*E*)-2,4-Nonadienal | | 0.4 | | | | | | | |
| 1726 | $\gamma$-Hexalactone | 6.4 | | | | | 1.6 | 1.5 | | 3.7 |
| 1751 | Carvone | | | | 1.3 | | | | | |
| 1763 | Naphthalene | | | | 0.7 | | | | | |
| 1779 | Methyl phenyl acetate | | 0.3 | | 3.4 | | | | | |
| 1793 | Methyl nicotinate | | | | | | | | | 3.8 |
| 1896 | Benzyl alcohol | 11.7 | 0.3 | 17.1 | 16.6 | | 11.6 | 9.7 | 28.9 | 13.3 |
| 1908 | Unknown** | | 0.5 | | | | 3.1 | 17.6 | | 6.4 |
| 1937 | Phenylethyl alcohol | 4.0 | 1.8 | 6.7 | 4.3 | 100 | 39.0 | 11.5 | 6.4 | 9.4 |
| 1957 | Benzene acetonitrile | | | | 0.8 | | | | | |
| 2088 | Methyl 2-methoxybenzoate | | | | 0.6 | | | | | |
| 2198 | Thymol | | | | 1.3 | | | | | |
| 2308 | Cinnamyl alcohol | | | | 0.9 | | | | | |
| | Total | 76.9 | 95.6 | 88.7 | 85.1 | 100 | 79.6 | 65.3 | 100 | 60.1 |

RRI: Relative retention indices calculated against *n*-alkanes, % calculated from TIC data.
tr: Trace (< 0.1%).
* Tentative identification from Wiley.
** Unknown; EIMS (70 eV): *m/z* (rel. int.) = 162 (6.9), 161 (13.4), 145 (96.2), 130 (14.8), 127 (15.1), 113 (27.9), 101 (35.8), 74 (15.4), 71 (100), 59 (20.1), 43 (76.1).

was reminiscent of RLL-1, however the major components were replaced by 6-methyl-5-hepten-2-one (11.9%), styrene (7.9%), calarene (7.1%) and benzyl alcohol (6.3%). The slight odor of the $CH_2Cl_2$ extract of *R. x sochadzeae* leaves (RSoL-2) was found to be due to phenylethyl alcohol, the only odorant detected in the headspace of this extract. RSoFr-2, the $CH_2Cl_2$ extract of the fruits of *R. x sochadzeae* was also characterized with a high percentage of phenylethyl alcohol (39.0%), accompanied with benzyl alcohol (11.6%), 2-ethylhexanol (7.0%) and 13 other minor volatiles. It is worth noting that the *n*-hexane-soluble fraction of the unripe fruits of *R. x sochadzeae* (RSoFr-1) was dominated by ethyl acetate (44.8%), a solvent never used in the present study. In order to exclude the possibility that EtOAc originates from the work-up procedure, we have analyzed the extraction solvents by GC-MS under the same conditions. No significant amount of EtOAc was detected in the solvents, indicating the natural presence of this chemical in the fruits.

6-Methyl-5-hepten-2-one (29.4%) and 2-ethylhexanol (24.4%) comprised the main constituents of the hexane-solubles of *R. ungernii* leaves (RUL-1). These two compounds, plus 8 less concentrated odorants shown in Table II represented 100% of the whole volatile material. The main constituents of the $CH_2Cl_2$ extract of the leaves (RUL-2) included an unknown compound (17.6%) with low molecular weight (*m/z* 162), as well as 2-(2-ethoxyethoxy) ethanol (12.8%), phenylethyl alcohol (11.5%) and benzyl alcohol (9.7%). The fresh flowers of *R. ungernii* had a light aromatic smell. Benzyl alcohol was the most predominant component (28.9%) in the headspace of the $CH_2Cl_2$ extract of these flowers (RUF-2).

The *n*-hexane extract of the leaves of the endemic species, *R. smirnovii* (RSL-1), showed some similarities to that of *R. ungernii* in its volatile profile. The headspace of the former extract consisted of 8 compounds, 6 of which are common with that of RUL-1 (Table II). 6-Methyl-5-hepten-2-one (21.7%), viridiflorol (15.4%) and 6-methyl-3,5-heptadien-2-one (14.6%) were the major constituents, representing the half (51.7%) of the whole volatile fraction (95.6%). Benzyl alcohol (13.3%), phenylethyl alcohol (9.4%) and 2-(2-ethoxyethoxy) ethanol (7.8%) appeared to be main components of the $CH_2Cl_2$ extract of the same material (RSL-2; Table III).

Essential oils of some *Rhododendron* species have been investigated. Mostly terpenes such as $\alpha$-humulene, caryophyllene, limonene, $\alpha$- or $\beta$-pinene comprise the main constituents of the essential oils (Shi, 1981; Ma *et al.*, 1983; Doss *et al.*, 1986; Belousov *et al.*, 1995). In our study, with few exceptions, mostly non-terpenic hydrocarbons, alcohols, esters and ketones were present as the major components. A direct comparison with the literature data, however, is not possible. The main poisonous constituents of *Rhododendron* plants, grayanotoxins (andromedotoxins), were not detected in any of the extracts. These diterpenes are unstable on heating and have low vapor pressure, hence they require derivatization (TMS) before the GC analysis (Terai and Tanaka, 1993). Andromedotoxin has been isolated from the waste material in the production of *Rhododendron* essential oils (Belova, 1971).

Despite their dangerous potential for the public health, none of *Rhododendron* species growing in Turkey has been subjected to a detailed chemical investigation. This is the first systematic chemical study performed on Turkish members of this genus. Furthermore, no report has appeared on the use of HS-SPME on the volatiles of this genus so far. The current study indicated the applicability and feasibility of SPME as an alternative in quick screening of both simple and complex mixtures of organic volatiles. Also it suggested the existence of differences in the volatile composition at species level, which certainly accounts for the perceived flavor differences of flowers and other anatomical organs.

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